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Monitoring stress levels with salivary alpha-amylase determination through a paper-based microfluidic analytical device

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Salivary alpha-amylase (AMY) serves as a vital biomarker for monitoring the human body's response to physical and psychological stress, acting as an indicator of system dysregulations and health conditions, such as ADHD in children. Saliva, contrary to blood, has a non-invasive collection, which is painless and does not need specialized operators, promoting a low-cost analysis of AMY levels that can be done frequently in an easier way. Combining the easy sampling of saliva with a simpler analysis method would be the perfect fit, as the traditional laboratory techniques for AMY determination, such as spectrophotometry, involve expensive equipment and long processing times. As so, a portable, low-cost and easy-to-use analytical tool is required to provide real-time monitoring of salivary AMY.

Therefore, in this work, a microfluidic paper-based analytical device (μ PAD) was developed for the determination of AMY in saliva samples, based on the enzymatic reaction between AMY and corn starch, where the starch is consumed by the salivary enzyme, followed by the colorimetric reaction between starch and iodine, resulting a blue colored product, in which intensity decreases with the increase of AMY quantity. The developed sensor consists of two layers of filter paper, where first, the AMY standards or sample solutions react with starch in the top layer for 5 minutes and, after that, an iodine solution prepared in ethanol is placed into the sampling holes to allow for the colorimetric reaction to occur for 15 minutes. This method can determine AMY levels in saliva between 10 to 50 U/mL, with a LOD of 2.53 U/mL and a LOQ of 8.42 U/mL, accounting for a dilution prior to analysis.

This represents a convenient tool for point-of-care analysis of salivary AMY and, subsequently, stress levels in humans, which can also be beneficial for veterinary analysis, since it can be used to monitor this parameter and general welfare in pigs and other animals.

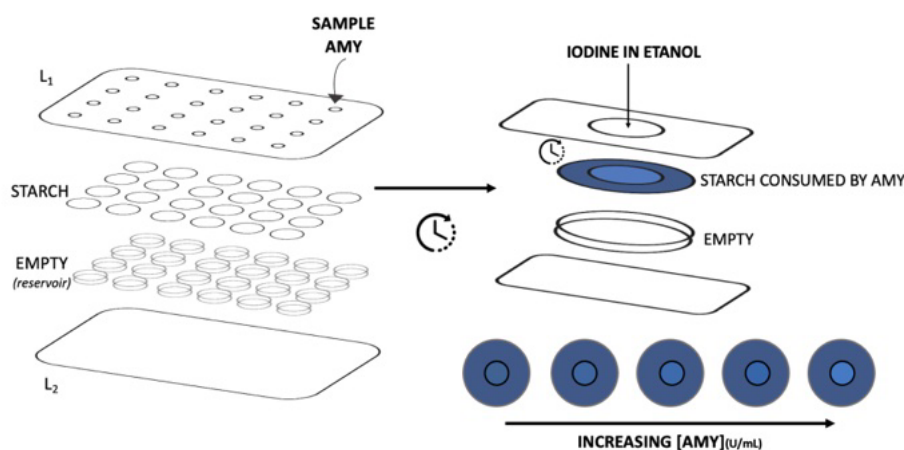


Figure 1: Schematic representation of the developed μ PAD for AMY determination.

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